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Supporting Information

Autoproteolytic Fragments Are Intermediates in the Oligomerization/ Aggregation of the Parkinson's Disease Protein Alpha-Synuclein as Revealed by Ion Mobility Mass Spectrometry

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Supporting Information

Materials and Methods

HPLC-MS analysis of αSyn oligomerization products

ESI-ion trap mass spectra were recorded on a Bruker Esquire 3000+ ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a direct infusion and ESI-LC-MS system. LC-MS experiments were carried out using an Agilent Technologies (Waldbronn, Germany) HP1100 liquid chromatograph for binary gradient elution (pump model G1312A), including an autosampler (G1313A) and a DAD (G1315 B) coupled to the ion trap mass spectrometer. A 150 mm x 4.6 mm x 3 μm Discovery RP-18 column was used for the separation of peptides. Mass spectra were recorded in the full scan mode, scanning from m/z 200 to 1500. Ion source parameters were 20 psi nebulizer gas and 9 L/min of drying gas, with a temperature of 300 °C. Tandem-MS experiments were carried out in the autofragmentation mode. Sequences of the extracted peptides were determined by subjecting the tandem- MS results to the NCBInr database search using the MASCOT programme.

Amino-Succinylation of alpha-synuclein

Chemical modification of proteins by amino-succinylation with succinic anhydride has been successfully used in the structure-function studies of several proteins, by reaction of succinic anhydride with lysine-ε-amino groups and the N-terminal α-amino group, converting them from basic to acidic protein derivatives[26]. Succinylation of αSyn was performed in 0.3 M sodium phosphate buffer, pH 7.5, by addition of increasing amounts (1 mg/mL) of a succinic anhydride solution in DMSO (0.5, 1, 2, 10, 50, 100-fold molar excess to protein) to the continuously stirred protein solution (1 mg/mL) over a period of 90 min, and the pH of the reaction mixture maintained by addition of 0.2 M NaOH. The reaction was allowed to proceed for 30 min at 25 °C and the protein solution then thoroughly dialyzed against water, followed by gel electrophoresis and mass spectrometric analysis. As shown in Figure S5A, B, the succinylation provided a protein derivative with a mass increase of 1700 Da corresponding to modification of all lysine residues (15) and the N-terminus, and the His50 residue; the fully succinylated αSyn does not show any oligomerization- aggregation, and only a dimer (Figure S5).

Table S1: αSyn Autoproteolytic truncation and degradation products observed by gel electrophoresis and identified by IMS-MS and MALDI-MS.

No. [a]	Spot No. [a]	m.w. gel [kDa] [b]	m.w. MS [c] [Da]	Sample description	Sequence [g]
1	a	16.7	14460.0 [d]	wt-αSyn	1-140
	a'	35.1	28919.6 [d]	wt-αSyn / dimer	1-140
	b	16.7	13706.6 [e]	wt-αSyn / N-terminal truncation	7-140
	c	14.5	12163.0 [d]	wt-αSyn / N-terminal truncation	14-133
	c'	25.9	25081.5 [f]	wt-αSyn / dimer of N-terminal truncation	
	d	12.3	7274.5 [d,e,f]	wt-αSyn / autoproteolytic fragment	72-140
	e	10.9	10436.0 [d]	wt-αSyn / N-terminal truncation	40-140
2		16.7	14329.4 [e]	αSyn V70A V71A T72A G73A V74A T75A	1-140
3		16.7	14245.4 [e]	αSyn V70G V71G T72G G73G V74G T75G	1-140
4		12.3	7365.3 [e]	αSyn(71-140) / chemical synthesis	71-140
5		12.3	7274.4 [e]	αSyn(72-140) / recombinant	72-140
6		17.4	14288.7 [e]	βsyn	1-140

[a]Syn sample/spot number corresponding to Figure 1.
[b]Mol. weight of bands observed by gel electrophoresis.
[c]Molecular mass of truncation/degradation products determined by mass spectrometry (MS)
[d]Molecular mass of truncation/degradation products determined by IMS-MS.
[e]Molecular mass of truncation/degradation products determined ESI-MS.
[f]Molecular mass of truncation/degradation products determined MALDI-TOF-MS.
[g]N-Terminal sequence determined by Edman sequencing (Applied Biosystems Procise-470 sequencer).

Table S2: Mass spectrometric characterization of electrophoretic bands: Tryptic peptides of monomer and dimer αSyn **1a** and **1a'** identified by a) LC/ESI-MS and b) MALDI-TOF-MS.

No.	Peptide sequence		Molecular ion observed	M_{exp}	M_{calc}
1	AKEGVVAAAEK	11 - 21	a) 536.7)$^{2+}$	1071.4	1071.6
2	EGVVAAAEK	13 - 21	a) (437.2)$^{2+}$	872.7	872.4
3	EGVVHGVATVAEKTK	46 - 60	a) (509.1)$^{3+}$	1523.4	1523.8
4	EGVVHGVATVAEK	46 - 58	a) (432.6)$^{3+}$	1295.1	1295.6
5	EGVLYVGSKTK	35 - 45	a) (950.8)$^{2+}$	1179.5	1179.6
6	EGVLYVGSK	35 - 43	a) (476.2)$^{2+}$	950.5	950.5
7	MDVFMK	1 - 6	a) (385.6)$^{2+}$	769.3	769.3
8	TVEGAGSIAAATGFVKK	81 - 97	a) (536.4)$^{3+}$	1606.2	1606.8
9	TKEQVTNVGGAVVTGVTAVAQK	59 - 80	a) (720.1)$^{3+}$	2157.2	2157.4
10	TVEGAGSIAAATGFVK	81 - 96	a) (740.2)$^{2+}$	1478.5	1478.6
11	EQVTNVGGAVVTGVTAVAQK	61 - 80	a) (643.6)$^{3+}$	1927.9	1928.1
12	TKEQVTNVGGAVVTGVTAVAQKT VEGAGSIAAATGFVKKDQLGK	59 -102	b) (4288.7)$^{1+}$	4287.7	4287.8
13	KDQLGKNEEGAPQEGILEDMPVDP DNEAYEMPSEEGYQDYEPEA	97 -140	b) (4959.1)$^{1+}$	4958.1	4958.1

Supporting Figures

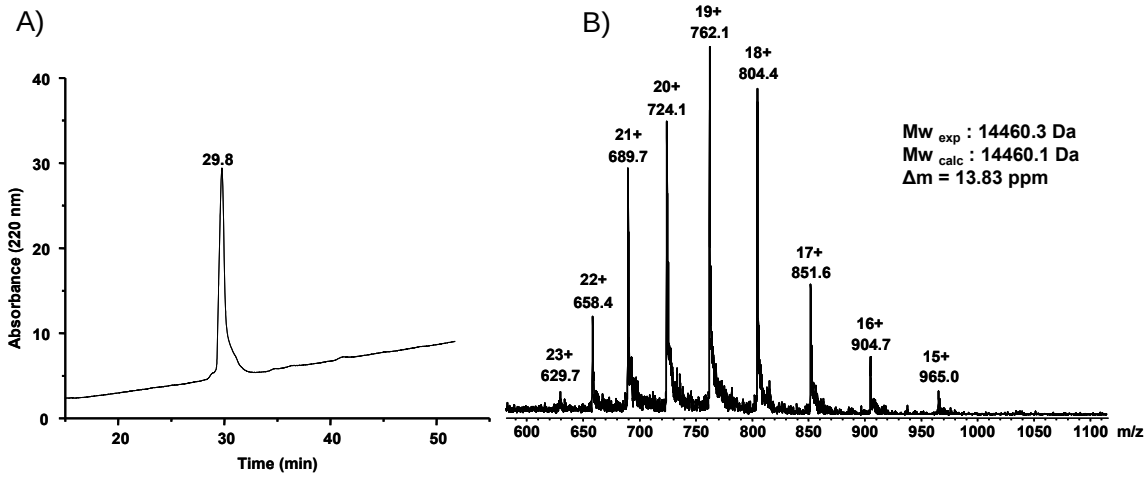

Figure S1: HPLC Purification (a) and ESI- mass spectrum (b) of freshly prepared recombinant *E. Coli* αSynuclein. See Materials and Methods for HPLC and MS analysis.

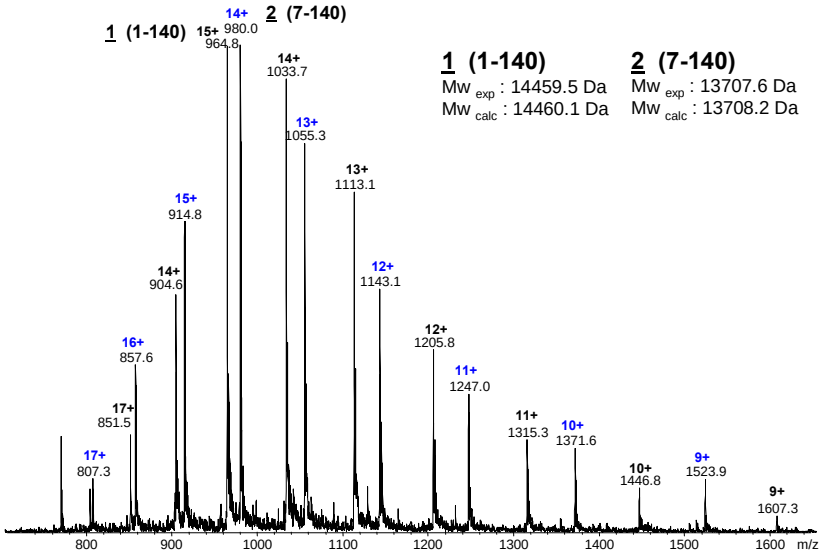

Figure S2: ESI- MS identification of N-terminal truncation product, αSyn(7 – 140), following 3 hrs incubation of αSyn in PBS buffer, pH 7.5. Edman sequence determination was performed with a Applied Biosystems Procise-470 sequencer as described)[22b].

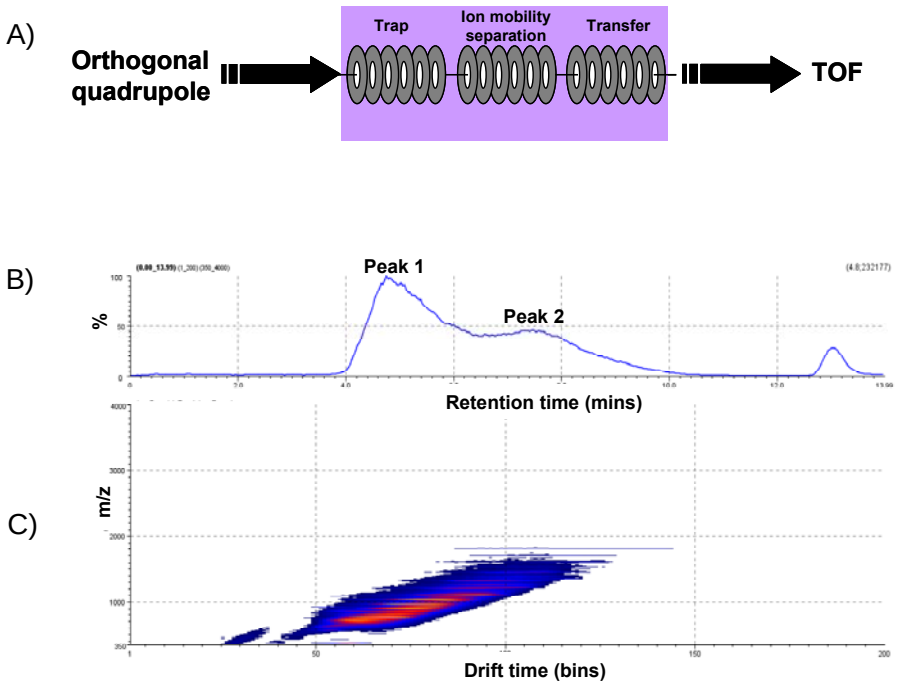

Figure S3: Scheme of the SYNAPT- ion mobility cell between the quadrupole analyzer and the orthogonal TOF analyzer[18a] (a); separation of peaks 1 and 2 (b); ion mobility drift time profile vs. m/z values of the IMS-MS analysis of the in vitro αSyn incubation mixture for 7 days (c).

^{72}TGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA^{140}

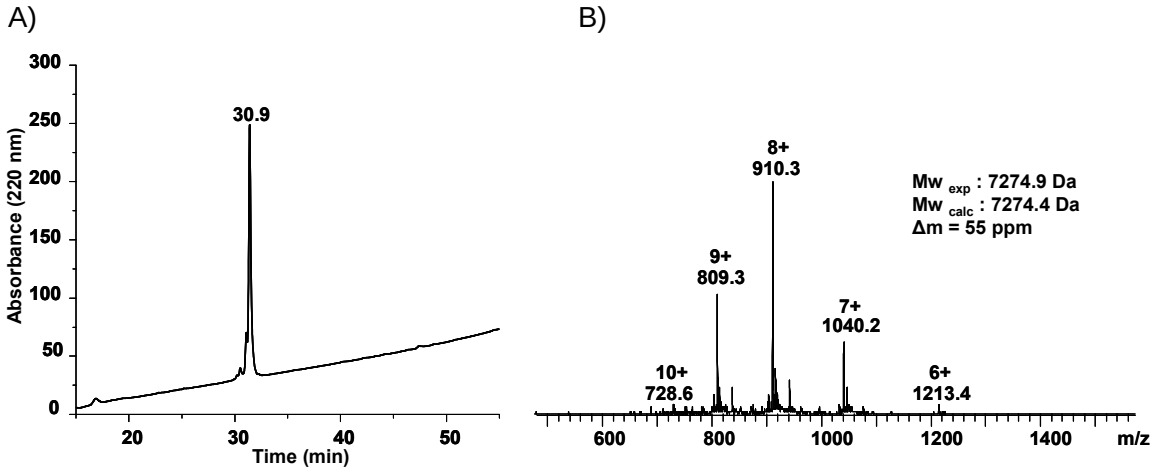

Figure S4: HPLC Purification (a) and ESI-mass spectrometric characterization (b) of recombinant αSyn(72-140) fragment expressed in E. Coli.

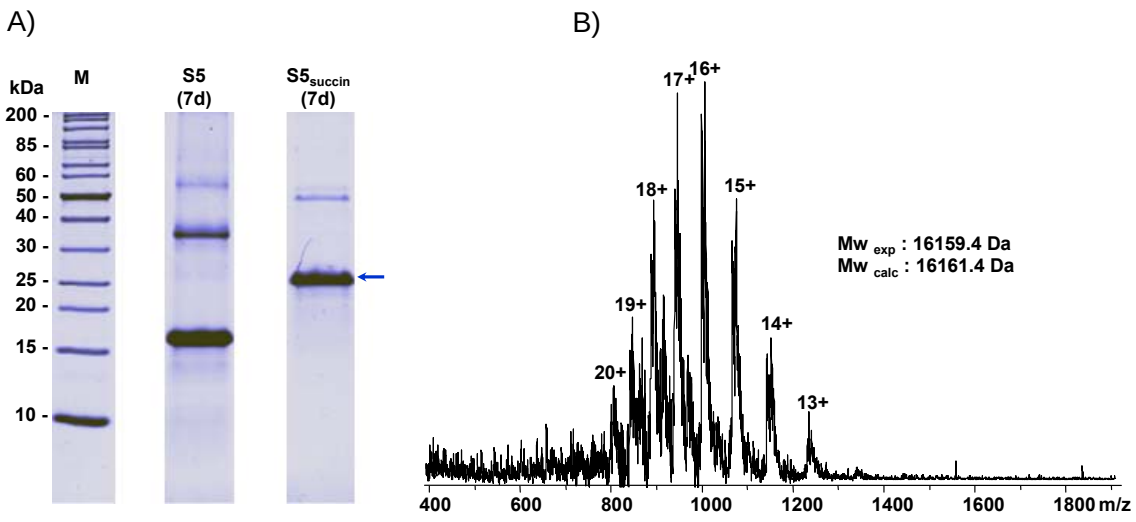

Figure S5: Gel electrophoretic and ESI-MS characterization of amino- succinylated αSyn **1**.